CYCLOTRICHIUM MEUNIERI SP. NOV. (PROTOZOA, CILIATA); CAUSE OF RED WATER IN THE GULF OF MAINE

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I. Introduction

During the warmest days of August, 1931, there were noticed in Frenchman Bay [1] on several occasions, great patches and lanes of red water. This has been known to occur in previous years but rarely so early as the fifth of August. These patches of red water were caused by swarms of small red animals, which appeared in layers from one to three meters beneath the water level. In spots these animals would be more crowded, thus varying the density of the patch as a whole and making the color vary from a brick to a blood red. Between the action of the wind and tide these patches would be streaked out in great lanes and irregular areas. On some days these patches would be more numerous than on others. As to the origin and ultimate fate of these areas of red water, the writer has no information.

A microscopic examination of a drop of sea water containing these organisms revealed hundreds of small red animals which moved rapidly in a zig-zag fashion reminding one of the characteristic movements of *Halteria*. On preliminary examination their appearance suggested that of trochophore larvæ; however, their incredible numbers and small size suggested that they were protozoa. Since the living animals died and disintegrated within one or two minutes upon exposure in a drop of water on a glass slip, no definite conclusion could be reached as to their morphology from a study of the living material.

Further study indicated that these animals were swarms of a ciliate belonging to the genus *Cyclotrichium* Meunier. I have named it *Cyclotrichium meunieri* sp. nov.

The writer wishes to express his appreciation to Mr. William Procter of Bar Harbor, Me., in whose laboratory this study was initiated; and to Dr. D. H. Wenrich for his many helpful suggestions and criticism of the manuscript.

[1] Frenchman Bay is that part of the Gulf of Maine which separates the northwest side of Mt. Desert Island from the mainland.

II. Technique

Quart jars of this red water were collected and allowed to stand in a cold place for about 20 minutes, in which time the organisms settled to the bottom. With a long pipette this sediment was collected and spurted into flasks half filled with warm (40° C.) fixative. Both Schaudinn's and Bouin's fixatives were used. After 30 minutes' fixation the animals were removed from the fixative with the aid of a centrifuge, washed and stored in 70 per cent alcohol. At the close of the summer, vials containing this fixed material were brought back to the University, where the slides were made and the study completed.

By mixing, on a cover-glass, a drop of this alcoholic sediment of fixed organisms with an equal amount of Mayer's egg albumen, spreading it carefully so that it would dry slightly, and finally flooding the cover-glass with absolute alcohol, these minute organisms were fastened to the cover-glass to facilitate their easy manipulation during staining. After 15 minutes in absolute alcohol the cover-slips were gradually transferred to water. Part of the material was stained with iron hæmatoxylin, Mayer's hæmalum, or Delafield's hæmatoxylin; and part by Feulgen's nucleal reaction.

It was found necessary to section some of this material, and in order to handle these small animals in paraffin a mixture of the alcoholic sediment and Mayer's egg albumen was placed in a concavity cut in a small block of preserved (70 per cent alcohol) liver, coagulated with absolute alcohol; and this block, with embedded protozoa, was then handled as a piece of tissue. By infiltrating in 67° paraffin and treating the block with ice water before cutting, sections of 4 μ could be cut with ease. The same stains were used for the sectioned material as for the whole mounts, with the exception that counterstains of orange G or eosine were used to demonstrate the cilia.

III. Observations

Cyclotrichium meunieri (Fig. 1), is almost oval, with the anterior end blunted and the posterior region slightly conoid.

Size.—This ciliate is relatively small. Twenty-five specimens selected at random from fixed material averaged 33 μ (25–42 μ) in length and 22 μ (18–34 μ) in width through the anterior region, whose diameter is somewhat greater than that of the posterior portion.

Cytostome.—The cytostome could not be definitely located; however, most of the specimens show, at the larger end, a depression (Figs. 1 and 2) slightly funnel-shaped and leading into the interior without any well-marked structures. This location of the cytostome agrees in general with that described by Meunier (1910) for *C. cyclokaryon;* and with descriptions given of the cytostomal regions of a number of species of *Cyclotrichium* described by Fauré-Fremiet (1924).

Ciliary Band.—A broad band of cilia is found about the middle. The fine, closely-set cilia are from 5 to 6 μ long and are found only in the depressed mid-region or ciliary band. Besides being depressed and ciliated, this band is further indicated by 52–60 striations which run parallel to the longitudinal axis. Each striation is made up of 5 to 9 granules; anteriorly the granules form a definite ring (Figs. 1 and 3, *A. G. R.*), while posteriorly they become slightly larger (Fig. 1). With iron hæmatoxylin these granules stain black, as do the metaplasmic granules of the endoplasm. With Delafield's hæmatoxylin or Mayer's hæmalum the metaplasmic granules fail to stain while the granules of the *ciliary band* are definitely outlined, thus indicating that the two types of granules are not the same. Because of the close association

EXPLANATION OF PLATE

Cyclotrichium meunieri sp. nov. All drawings have been made at a magnification of 2900 diameters and reduced about two-fifths in printing. The animals have been fixed with Bouin's fluid, stained in Heidenhain's hæmatoxylin, with the exception of those shown in Figs. 7 and 8 which were treated for the Feulgen reaction.

Abbreviations

A.G.R., anterior granular ring
B.G., basal granules
C., cilia
C.B., ciliary band
C.Bu., ciliary bundles
C.C., compound cilia
Ch., chromatin
C.P., chromatophore platelets
Cy., cytostome
E., endoplasm with inclusions
E.V., extra-nuclear vesicle
L.S., longitudinal striations
M., macronucleus
m., micronucleus
Mc., metaplastids
P., pellicle
P.B., pyrenoid body
Pl., plastin
S.S., shrinkage space or artifact

Explanation of Figures

1. Side view of *Cyclotrichium meunieri.* Chromatophore platelets and pyrenoid bodies do not stain in the whole mounts.

2. Sagittal section showing arrangement of chromatophore platelets, pyrenoid bodies, etc.

3. Sagittal section through region of ciliary band showing the arrangement of the basal granules.

4. A diagram of the possible arrangement of cilia in compound units to explain structures as shown in Fig. 6.

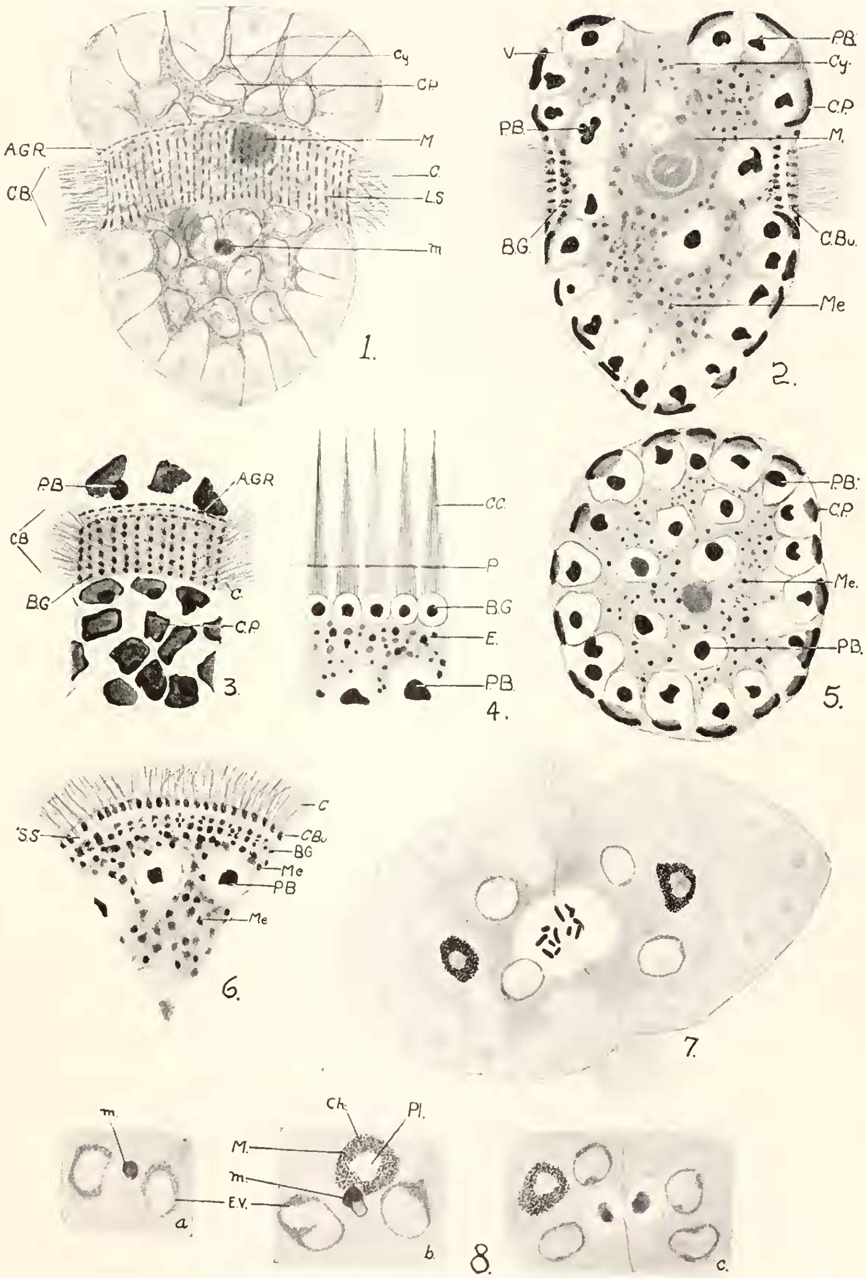
5. Cross section through anterior region.

6. Cross section through region of ciliary band. Cilia are finer and more numerous than could be shown in drawing.

7. Early telophase in dividing individual; six chromosomes are seen in each daughter.

8. Nuclear complexes showing variations. (*a*) micronucleus and two extra-nuclear vesicles; (*b*) micro- and macronucleus and two extra-nuclear vesicles; (*c*) a stage somewhat later than that shown in Fig. 7. Macronucleus is seen in only one of the daughter cells.

PLATE I


Cy
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5.
C
CBu
SS
BG
Me
PB
Me
6.
7.
m.
Ch.
Pl.
M.
m.
E.V.
a
b
8.
c.

of these granules of the striations with the cilia, I have designated them as basal granules.

The uneven movement of the living animal suggests the presence of cirri or membranelles; however, since the sectioned material showed only these exceedingly fine and numerous cilia and their large associated (compound?) basal granules (Fig. 3, *B. G.*), it would seem that we have in the living condition a system of compound cilia which at death separate into their component units (Fig. 6, *C.*). A diagrammatic interpretation of these compound cilia is shown in Fig. 4. Further evidence for this arrangement is presented by the staining reaction. With iron hæmatoxylin the points of insertion of the cilia stain after the manner of a clump of fibrils (Fig. 6, *C.*, *Bu.*), suggesting definite bunches or bundles of cilia.

Ectoplasm.—Beneath a thin, smooth pellicle, completely shielding the endoplasm, is a series of irregularly concave chromatophore platelets (Fig. 2, *C. P.*), each with an associated pyrenoid body (Fig. 2, *P. B.*); the whole being inclosed by a large vacuole. The red color of *C. meunieri* is doubtless due to the presence of a hæmatochromatous substance localized in the chromatophore platelets, which are possibly of an amylaceous nature. These platelets stain deeply with iron hæmatoxylin but not at all with Mayer's hæmalum. That these platelets have a definite body is demonstrated by the fact that they are often torn from their place due to the impact of the knife during sectioning. In the region of the ciliary band the platelets are missing while the pyrenoid bodies with their vacuoles remain (Fig. 2).

Endoplasm.—After staining sections with iron hæmatoxylin the endoplasm is crowded with many darkly staining bodies. These are designated metaplasmic granules, for they are doubtless associated with the metabolic processes. Because of the abundance of these metaplasmic granules, the nuclear apparatus could be demonstrated successfully only by the aid of the Feulgen technique.

A typical ciliate nuclear complex is present. The macronucleus is slightly irregular and demonstrates a definite core of plastin (Fig. 8*b*, *M.*) surrounded by a layer of granular chromatin. The micronucleus is small, sometimes vesiculated (Fig. 8*b*, *m*). Besides the macro- and micronucleus there are found one or more bodies, irregular in shape and staining but slightly with Feulgen's reagent, which are designated as extra-nuclear vesicles (Fig. 8, *E. V.*). These extra-nuclear vesicles are always present and seem to be either the formative or degenerative stage in the development of the macronucleus.

A number of dividing individuals were studied, and in one (Fig. 7) showing an early telophase stage in the division of the micronucleus six chromosomes could be counted in each daughter nucleus. A somewhat later stage than this is shown in Fig. 8*c*; in this case only one daughter individual received a definitive macronucleus, the other had but the extranuclear vesicles.

Occurrence.—Since single individuals of *C. meunieri* have been found in sea water taken either from the storage tanks of the laboratory, or among the material from plankton hauls; it seems reasonable to look upon this species as a member of the protozoan fauna of this region. Its sudden appearance in swarms among the surface plankton of the bay must be correlated with the periodic enrichment of the water by the nitrogen-bearing algæ whose numbers increase during periods of warmth and excessive sunshine; all of which factors tend to make areas of the bay excellent culture media for these red water ciliates. In the present instance the summer had been warmer than usual, particularly during the last of July and the first week of August.

IV. Discussion

Many organisms are known, under favorable conditions, to multiply in such numbers as to discolor great bodies of water. Martin and Nelson (1929) review this subject and give instances of red water occurring in Delaware Bay due to the swarming of *Amphidinium fusiforme*. Kofoid and Swezy (1921) record the occurrence of swarms of *Gonyaulax polyhedra* as being the most frequent cause of red water along the Pacific coast.

I have placed the ciliate causing red water in the Gulf of Maine in the genus *Cyclotrichium* Meunier because it seems to resemble very closely a ciliate of rare occurrence in the plankton hauls from Barents Sea. Meunier (1910) established this genus for *C. cyclokaryon* but figured a number of ciliates as *Cyclotrichium* Sp? because their poor preservation would not permit further classification. Those ciliates were included in the genus *Cyclotrichium* which had a ciliary band or belt in an equatorial depression which divided the body into two subspherical halves. Making allowance for the poor fixation of his material, the organisms which he described under the name of *Cyclotrichium* Sp? may be likened to those found off the coast of Maine which I have named *C. meunieri*.

The Maine fishermen recognize this red water as a source of food for the herring sardine and it is said that when the red water is present in the herring's intestines they become unfit for sale. Mackerel are also reported by the fishermen as sometimes containing " red feed " in their digestive tracts. It would seem that " red water " and " red feed " are due to two different organisms. Inquiring into this condition further, the writer corresponded with Dr. A. G. Huntsman, Director of the Atlantic Biological Station at St. Andrews, mentioning these occurrences. Dr. Huntsman replied as follows:

"In the St. Andrews region we have never seen the water coloured crimson by the form you mention. In warm summers the warmest strip near the center of the upper end of Passamaquoddy Bay sometimes becomes decidedly reddish and this has been found due to large numbers of different kinds of Tintinnoids which have been studied by Professor J. N. Gowanlock, although his report has not yet been published. In the St. Andrews region the term 'red feed' is given to copepods as they occur in the stomach of herring, particularly the young herring or sardine. The most abundant form is *Calanus* and at times the swarms of this species give a reddish cast to small areas of the water."

To my knowledge *Cyclotrichium meunieri* is the first holotrichous ciliate to be associated with the appearance of red water in the ocean.

V. Summary

1. Red water in Frenchman Bay is caused by the swarming of a small red ciliate, *Cyclotrichium meunieri* sp. nov.

2. This organism is ovoid, about 33 μ long, and has in a wide depression about its middle a band of fine cilia. It has been suggested that compound cilia may be present in the living animals. The endoplasm is well shielded by a peripheral series of chromatophore platelets, each with an associated pyrenoid body.

3. Six chromosomes were observed in each of the daughter micronuclei in an early telophase of division.

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